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Comparison of the antiplasmodial and falcipain-2 inhibitory activity of β-amino alcohol thiolactone-chalcone and isatin-chalcone hybrids

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ABSTRACT

The synthesis and biological evaluation of two novel series of natural-product-like hybrids based on the chalcone, thiolactone and isatin scaffolds is herein described. Results for a 36-member β-amino alcohol triazole library showed that the thiolactone-chalcones, with IC_{50}s ranging from 0.68 to 6.08 μM, were more active against W2 strain *Plasmodium falciparum* than the isatin-chalcones with IC_{50}s of 14.9 μM or less. Also of interest is falcipain-2 inhibitory activity displayed by the latter, whereas the thiolactone-chalcones lacked enzyme inhibitory activity.

The reported decrease in the number of New Chemical Entities (NCEs) introduced annually coupled with the increasing pace at which resistance against established anti-infective drugs develops presents a precarious situation especially for resource-poor countries with high endemicity for infectious diseases. Thus, there is an increasing need for new, potent anti-infective agents.

One focus of our research programme is on the design, synthesis and biological evaluation of hybrid compounds to identify novel anti-infective agents. The rationale for this approach derives from the expeditious SAR study offered through pharmacophore-rich compound libraries.[1] Further appeal stems from the reduced propensity for resistance development, the improved toxicity and pharmacokinetic profile and enhanced and/or new properties displayed by the hybrids so obtained.[2] Another important, although not often referred to, advantage offered is the potential avoidance of unfavourable drug–drug interactions which may occur in combination therapy or in the treatment of HIV, malaria and TB co-infections.

This Letter reports on the synthesis and antiplasmodial activity of two novel series of natural-product-like hybrids. Incorporated in their design is the β-amino alcohol moiety, a known antimalarial pharmacophore,[3] and the solubility enhancing 1,2,3-triazole ring system which is accessible via the Huisgen 1,3-dipolar cycloaddition reaction and functions as linker in the envisaged hybrids. To our knowledge hybrids of this nature have not been reported previously. In an attempt to elucidate their mode of action the hybrids were evaluated for inhibitory activity against the cysteine protease falcipain-2, a validated target for antimalarial chemotherapy.

For guidance in hybrid design, we relied on previous SAR studies and the thiolactomycin (**1**)-FabB model. One of the key drug-receptor interactions revealed by this model is that the C-4 hydroxyl group of **1** (Fig. 1) extends into an incompletely filled pantetheine pocket.[4] Furthermore, a study revealed that modification of the latter group, on C-5 derivatized analogues of **1**, afforded

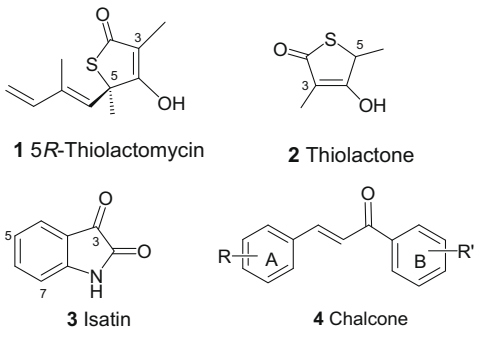

Figure 1. Natural product and natural product-like scaffolds used in hybrid construction.

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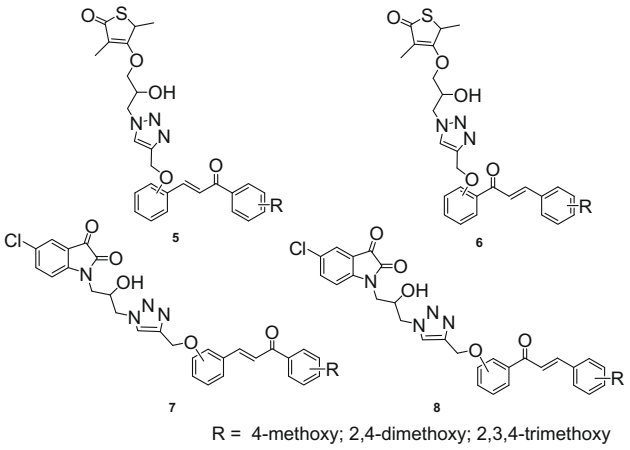
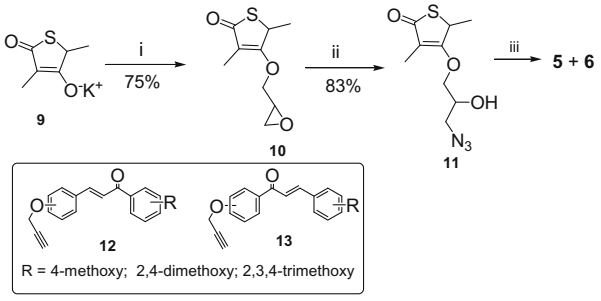


Figure 2. β-Amino alcohol hybrids **5–8**.



Scheme 1. Reagents and conditions: (i) (±)-Epichlorohydrin (excess), 130 °C, 7 h; (ii) NaN_3 (5 equiv), NH_4Cl (3 equiv), $MeOH/H_2O$ (8:1), 25 °C, 48 h; (iii) **12** (for **5**, 1.1 equiv) or **13** (for **6**, 1.1 equiv), $CuSO_4{\cdot}5H_2O$ (5 mol %), sodium ascorbate (10 mol %), CH_2Cl_2/H_2O (1:1), 25 °C, 16 h.

analogues which displayed a 143-fold increase in antimalarial activity relative to the parent compound **1**—a selective type II FAS inhibitor.[5] It was therefore anticipated that modification at the C-4 hydroxyl group of **2** could provide analogues with enhanced antimalarial activity. Derivatives of isatin **3** reportedly display a wide spectrum of activity which includes the inhibition of cysteine and serine proteases.[6] Other attractive features of **3** include its non-peptidic nature and the ease with which it can be synthetically manipulated, making it the ideal template onto which bioactiphores can be appended.[7] The rationale behind utilising the 5-chloroisatin derivative in target hybrids **7** and **8** stems from reports on the modulating effect of the chlorine atom on the chemical and biological properties of compounds.[8] Its presence in bioactive natural and synthetic drugs, for example vancomycin, griseofulvin, clindamycin and especially chloroquine, is noteworthy.

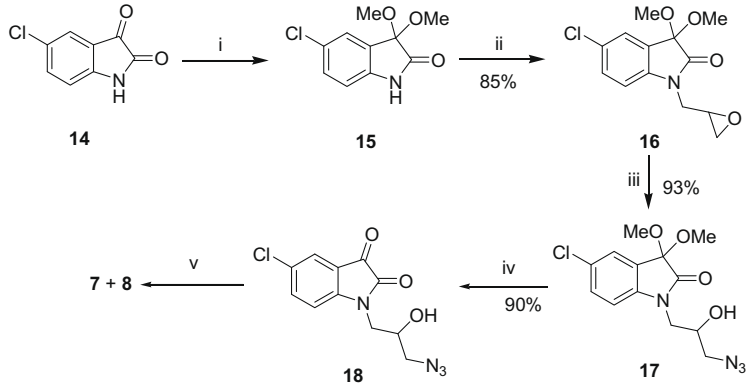
As shown in Figure 2, the chalcone scaffold is a common monomer in the envisaged hybrids **5–8**. Chalcones **4** are of considerable interest in drug discovery because of the diverse biological activities displayed by derivatives[9] and the ease and simplicity of their synthesis. As antimalarials, they may exert their effect via inhibition of cysteine proteases[10] or new permeability pathways.[11] Moreover, this scaffold allows for the systematic variation of substituents and/or substitution patterns on the aromatic rings A and B for SAR investigation. Selection of the methoxy substituent is on the basis of previous SAR which revealed that alkoxylated chalcones are important for antimalarial activity.[10,11]

The envisaged 18-member thiolactone-chalcone hybrid library is organized into two series **5** and **6**, as depicted in Figure 2. The aromatic ring A of **5** has the triazole moiety as a substituent attached at either the *ortho*-, *meta*- or *para*-position, whereas ring B is methoxylated to varying degrees. A similar arrangement holds for **6** with the only difference being that the substituents on rings A and B are interchanged. SAR studies set out to investigate the effect on antimalarial activity of (i) the number of methoxy substituents on ring B and ring A for **5** and **6**, respectively, (ii) the position of attachment of the triazolic moiety on ring A and ring B for **5** and **6**, respectively, and (iii) the importance of substitution on ring A/B for activity. The isatin-chalcone hybrids **7** and **8** were subjected to a similar SAR study.

Synthesis of the thiolactone hybrids **5–6** is outlined in Scheme 1. The intermediate **10** was obtained by reacting the potassium salt of thiolactone **9** with a large excess of (±)-epichlorohydrin at 130 °C for 7 h under a nitrogen atmosphere.

For the azidolysis of **10** we employed a modification of the classical, non-chelating conditions.[12] It involved the reaction of 1 equiv of **10**, 5 equiv of NaN_3 and 3 equiv of NH_4Cl—which acts as both proton source and buffer—in aqueous alcohol at room temperature. The latter method afforded **11** regioselectively in 83% yield.

Used in the final step of the synthesis of racemic **5** and **6**, is a procedure reported by Lee et al.[13] Following this procedure 1 equiv of the azide **11**, 1.1 equiv of the acetylene **12** (**13** for target molecule **6**), 5 mol % of $CuSO_4{\cdot}5H_2O$ and 10 mol % of sodium ascorbate was stirred in CH_2Cl_2/H_2O (1:1) at 25 °C. Scheme 2 outlines the synthesis of isatin-chalcone hybrids **7–8**. The direct alkylation of 5-chloroisatin **14** with (±)-epichlorohydrin did not yield the desired product. Synthesis of **7–8** therefore commenced with the acetalization of **14**. The protocol employed involved heating under



Scheme 2. Reagents and conditions: (i) $CH(OMe)_3$ (30 equiv), *p*-$TsOH_{cat}$, MeOH, reflux, 48 h; (ii) (±)-epichlorohydrin (5 equiv), KF/Al_2O_3 (5 equiv), DCM, 25 °C, 20 h; (iii) NaN_3 (5 equiv), NH_4Cl (3 equiv), $MeOH/H_2O$ (8:1), 25 °C, 16 h; (iv) 10% HCl, acetone, 25 °C, 3 h; (v) **12** (for **7**, 1.1 equiv) or **13** (for **8**, 1.1 equiv), $CuSO_4{\cdot}5H_2O$ (5 mol %), sodium ascorbate (10 mol %), CH_2Cl_2/H_2O (1:1), 25 °C, 3 h.

Table 1
In vitro antimalarial activity against chloroquine-resistant (W2) strains of *P. falciparum* and recombinant falcipain-2 inhibitory activity for **5–8**

Compd	Position	R	rec-FP-2,[a] IC_{50} (μM)	W2, IC_{50} (μM)
1			>100	>20
2			>100	>20
3			>100	>20
5a	*ortho*	4-OMe	>100	2.31
5b	*ortho*	2,4-diOMe	>100	2.08
5c	*ortho*	2,3,4-triOMe	>100	1.14
5d	*meta*	4-OMe	>100	2.43
5e	*meta*	2,4-diOMe	>100	1.64
5f	*meta*	2,3,4-triOMe	>100	2.00
5g	*para*	4-OMe	>100	3.12
5h	*para*	2,4-diOMe	>100	1.54
5i	*para*	2,3,4-triOMe	>100	5.04
6a	*ortho*	4-OMe	>100	2.64
6b	*ortho*	2,4-diOMe	>100	2.14
6c	*ortho*	2,3,4-triOMe	>100	1.72
6d	*meta*	4-OMe	>100	3.69
6e	*meta*	2,4-diOMe	>100	3.51
6f	*meta*	2,3,4-triOMe	>100	0.68
6g	*para*	4-OMe	>100	6.08
6h	*para*	2,4-diOMe	28.5	3.10
6i	*para*	2,3,4-triOMe	>100	0.81
7a	*ortho*	4-OMe	25.44	>20
7b	*ortho*	2,4-diOMe	15.58	>20
7c	*ortho*	2,3,4-triOMe	90.47	6.10
7d	*meta*	4-OMe	10.29	5.75
7e	*meta*	2,4-diOMe	6.80	3.17
7f	*meta*	2,3,4-triOMe	11.49	2.18
7g	*para*	4-OMe	10.84	10.61
7h	*para*	2,4-diOMe	ND	9.73
7i	*para*	2,3,4-triOMe	16.62	10.98
8a	*ortho*	4-OMe	15.96	7.79
8b	*ortho*	2,4-diOMe	15.04	2.09
8c	*ortho*	2,3,4-triOMe	15.33	6.21
8d	*meta*	4-OMe	9.91	3.57
8e	*meta*	2,4-diOMe	10.61	2.95
8f	*meta*	2,3,4-triOMe	16.73	2.57
8g	*para*	4-OMe	15.89	14.90
8h	*para*	2,4-diOMe	18.28	14.52
8i	*para*	2,3,4-triOMe	25.06	>20
11			>100	>20
17			>100	>20
Chloroquine			—	0.0694
E64			0.04733	~3

[a] Rec-FP-2 = recombinant falcipain-2.
[b] ND = not determined.

reflux a mixture of 1 equiv of **14** and 30 equiv of anhydrous trimethyl orthoformate in anhydrous MeOH in the presence of a catalytic amount of *para*-toluene sulfonic acid for 48 h. With **15** in hand, the next step involved synthesis of the epoxide **16**. This was achieved by the reaction of **15** with 5 equiv of (±)-epichlorohydrin in the presence of 5 equiv of KF-Al_2O_3 in anhydrous CH_2Cl_2 at 25 °C.

Synthesis of the azide intermediate **17**, proceeded as mentioned earlier. Acid hydrolysis of **17** afforded **18** which participated in the ligand-free Cu(I) catalyzed 1,3-cycloaddition protocol described earlier. As shown in Scheme 2, hybrid **7** was obtained by reaction of the azide **18** with the acetylene **12** in the presence of 5 mol % of $CuSO_4 \cdot H_2O$ and 10 mol % of sodium ascorbate in 4 mL DCM/H_2O (1:1) at room temperature. Synthesis of **8** proceeded in a similar fashion with the acetylenic chalcone **13**.

All the hybrids **5–8** gave ^1H, ^{13}C NMR, IR, HRMS and elemental analysis data consistent with the proposed structures.

Hybrids **5–8** were evaluated for growth inhibitory activity against the W2 chloroquine-resistant strain of *Plasmodium falciparum*[14] as well as for inhibitory activity against recombinant falcipain-2.[14] The results obtained are summarised in Table 1.

The β-amino alcohol thiolactone-chalcone hybrids **5–6** showed promising antiplasmodial activity with IC_{50}s ⩽ 6.08 μM against the W2 strain. However, relative to the control drug chloroquine, these activities were still poor. The most interesting compound identified is the *meta*-substituted, trimethoxylated derivative **6f** [IC_{50} = 0.68 μM]. Molecules **5–6**, with the exception of **6h**, did not show falcipain-2 inhibitory activity at the maximum concentration tested. This result suggests that impairment of other parasite-pathways or targets is responsible for antimalarial activity. Results for compounds **5–6** also indicate that antiplasmodial activity against the W2 strain increases with an increase in the number of methoxy substituents on the chalcone moiety, as exemplified by **5a–c** and **6a–i** and is consistent with earlier reports.[10,11] The enhanced parasite inhibitory activity displayed by **6f** and **6i** indicates a preference for methoxy substituents on ring A, whereas the activities of compounds **5f** and **6f** suggest a preference for *meta* substitution as far as the attachment of the triazole moiety to the chalcone scaffold is concerned.

The isatin-chalcone hybrids **7–8** also displayed promising activity but were generally less active than the thiolactone-chalcone hybrids **5–6**. Moreover, all compounds in the series **7–8** inhibited falcipain-2 activity, although they were several 100-fold less active than the cysteine protease inhibitor E64. However, E64 shows fairly weak antiplasmodial activity against W2 (IC_{50} ~3 μM), presumably due to poor cell penetration. The results further show that for falcipain-2 inhibitory activity *meta* substitution is preferred, and that increased methoxylation does not generally enhance activity (Table 1). Failure to correlate enzyme inhibitory activity with growth inhibitory activity led us to conclude that cysteine protease inhibition is not the primary mode by which these hybrids (**7**, **8**) exert their antimalarial effect.

In conclusion a novel series of β-amino alcohol hybrids which displayed encouraging antiplasmodial activity has been designed and synthesized. The results demonstrate the potential for hybridization as an antimalarial drug discovery tool as evidenced by the lack of activity of the advanced intermediates **11**, **17**, **12–13**[15] and parent drugs (**1** and **3**) compared to the low micromolar activity of the hybrids **5–8**. This study also confirmed the interaction capabilities of the isatin scaffold with the thiols of cysteine proteases.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.017.

References and notes

1. Biot, C.; Chibale, K. *Infect. Disord. Drug Targets* **2006**, *6*, 173.
2. (a) Barbachyn, M. R.. In *Ann Rep Med Chem*; Macor, J. E., Ed.; Elsevier Inc.: USA, 2008; Vol. 43, p 281; (b) Tietze, L. F.; Bell, H. P.; Chandrasekhar, S. *Angew. Chem.,*

Int. Ed. **2003**, *42*, 3996; (c) Vennerstrom, J. L.; Arbe-Barnes, S.; Brun, R.; Charman, S. A.; Chiu, F. C. K.; Chollet, J.; Dong, Y.; Dorn, A.; Hunziker, D.; Matile, H.; McIntosh, K.; Padmanilayman, M.; Santo, T. J.; Scheurer, C.; Scorneaux, B.; Tang, Y.; Urwyler, H.; Wittlin, S.; Charman, W. N. *Nature* **2004**, *430*, 900.
3. Pérez-Silanes, S.; Berrade, L.; García-Sánchez, R. N.; Mendoza, A.; Galiano, S.; Pérez-Solórzano, B. M.; Nogal-Ruiz, J. J.; Martínez-Fernández, A. R.; Aldana, I.; Monge, A. *Molecules* **2009**, *14*, 4120.
4. Price, A. C.; Choi, K. H.; Heath, R. J.; Li, Z.; White, S. W.; Rock, C. O. *J. Biol. Chem.* **2001**, *276*, 6551.
5. Jones, S. M.; Urch, J. E.; Brun, R.; Harwood, J. L.; Berry, C.; Gilbert, I. H. *Bioorg. Med. Chem.* **2004**, *12*, 683.
6. (a) Lyer, R. A.; Hanna, P. E. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 89; (b) Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3527; (c) Daisley, R. W.; Shah, V. K. *J. Pharm. Sci.* **1984**, *73*, 407; (d) Pandeya, S. N.; Sriram, D.; De Clerq, E.; Panne-Couque, C.; Witurouw, M. *Indian J. Pharm. Sci.* **1998**, *60*, 207.
7. Chiyanzu, I.; Clarkson, C.; Smith, P. J.; Lehmann, J.; Gut, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem.* **2005**, *13*, 3249.
8. Naumann, K. *J. Prakt. Chem.* **1999**, *341*, 417.
9. (a) Nielsen, S. F.; Bosen, T.; Larsen, M.; Schonning, K.; Kromann, H. *Bioorg. Med. Chem.* **2004**, *12*, 3047; (b) Go, M. L.; Wu, X.; Liu, X. L. *Curr. Med. Chem.* **2005**, *12*, 483; (c) Chen, M.; Theander, T. G.; Christensen, B. S.; Hviid, L.; Zhai, L.; Kharazmi, A. *Antimicrob. Agents Chemother.* **1994**, *38*, 1470; (d) Lin, Y.-M.; Zhou, Y.; Flavin, M. T.; Zhou, L.-M.; Nie, W.; Chen, F.-C. *Bioorg. Med. Chem.* **2002**, *10*, 2795.
10. Ring, C. S.; Sun, E.; McKerrow, J. H.; Lee, G. K.; Rosenthal, P. J.; Kuntz, I. D.; Cohen, F. E. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3583.
11. Go, M. L.; Liu, M.; Wilairat, P.; Rosenthal, P. J.; Saliba, K. J. *Antimicrob. Agents Chemother.* **2004**, *48*, 3241.
12. Swift, G. A.; Swern, D. *J. Org. Chem.* **1967**, *32*, 511.
13. Lee, B.-Y.; Park, S. R.; Jeon, H. B.; Kim, K. S. *Tetrahedron Lett.* **2006**, *47*, 5105.
14. Greenbaum, D. C.; Mackey, Z.; Hansell, E.; Doyle, P.; Gut, J.; Caffrey, C. R.; Lehman, J.; Rosenthal, P. J.; McKerrow, J. H.; Chibale, K. *J. Med. Chem.* **2004**, *47*, 3212.
15. Hans, R. H.; Guantai, E. M.; Lategan, C.; Smith, P. J.; Wan, B.; Franzblau, S. G.; Gut, J.; Rosenthal, P. J.; Chibale, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 942.